PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Exposure to benzene and childhood leukaemia: a pilot case-control study
AUTHORS	Lagorio, Susanna; Ferrante, Daniela; Ranucci, Alessandra; Negri, Sara; Sacco, Paolo; Rondelli, Roberto; Cannizzaro, Santina; Torregrossa, Valeria; Cocco, Pierluigi; Forastiere, Francesco; Miligi, Lucia; Bisanti, Luigi; Magnani, Corrado

VERSION 1 - REVIEW

REVIEWER	David Pyatt, PhD Principal, Co-Founder SUmmit Toxicology Adjunct Professor CU School of Pharmacy and School of Public Health
REVIEW RETURNED	04-Dec-2012

GENERAL COMMENTS	Overall: This manuscript describes a small, preliminary research project exploring the feasibility of conducting a case-control study of childhood leukemia with quantification of benzene exposures for the cases and controls. This study also evaluated biomarkers of exposure and well as collected actual air measurements for the participants. This is a welcome change from previously published studies on this topic and is a far superior approach (especially compared to relying on indirect and surrogate measures such as traffic density or proximity to gas stations, etc). Therefore, the goal of this pilot study is very important. Overall, this makes a reasonable scientific contribution with enough merit to warrant its publication and more importantly, to push forward with the expanded analysis. The goal(s) of the project was clearly laid out and the conclusions were supported by the data. General Comments: 1) While the concept and conduct of the study was good, the actual manuscript was poorly written. The bullet point style utilized was disorientating. At times, it seemed more like I was reading an outline of the actual manuscript than a finished text. It was very choppy to read and consequently, difficult to follow. I am not familiar with the 'open' journal format, so perhaps this is acceptable, but I would assume that any manuscript suitable for publication should be written in a more cohesive fashion. 2) There is a very important study just published that should be considered carefully by these investigators [1]. This pooled analysis from three very large nested case control studies has direct relevance to this project. In that analysis, MDS (myelodysplasia or myelodysplastic syndrome) was the endpoint most closely

	associated with low benzene exposures. I think moving forward, any study evaluating the health effects of benzene in children or anyone else, will have to at least consider that MDS might be a more (or equally) relevant endpoint, particular for low environmental exposures. 3) Another debate that has been ongoing for some time is the most relevant exposure metric for estimating the risk of adverse effects from benzene exposures. In this paper, cumulative exposure is the only factor evaluated. That is the most straightforward exposure metric to quantitate, but the authors should at least discuss the other metrics available and the data supporting their inclusion in any quantification of benzene. For example, in the Schnatter et al (2012) investigation, 'peak' exposures were highly correlated with MDS risk. There are other studies that also suggest that cumulative exposure is not the only meaningful consideration with regard to the exposure history. 4) As indicated by the authors, there is general scientific consensus that AML (or ANLL) can be caused by high exposures to benzene. However, the data surrounding benzene exposure and ALL, particularly in children, is essentially non-existent. As this study only has 4 childhood AML cases, it really does not inform on the issue of environmental levels of benzene and childhood AML risk (or MDS), which might be the most important question to address. 5) Lastly, it is also generally agreed that urinary biomarkers for benzene exposure become less reliable at environmental exposure levels. These authors have a fairly good correlation between S-PMA and benzene exposure, but this is not widely collaborated with several other environmental papers. Benzene in blood on the other hand, has been tightly correlated with exposure levels, even very low ones, and while the technique is obviously more invasive, it seems to be a more robust biomarker [2, 3]. As such, it should at least be mentioned in the manuscript. Specific Comments: • P4, L7, The term "limited evidence" is written twice • P4, L9, This sentence is unclear. Exposure to benzene causes what type of leukemia? Also, what exactly are 'relatively high, lifetime environmental exposures'? This sentence as written is difficult to interpret. • P4, L38, The entire concept of correlation with magnetic fields confusing. Why this even being considered? There has been a lot of effort trying to understand the potential relationship between EMF and childhood leukemia risk. Is this why the reader should care about this? The rationale for investigating this potential connection is not clear (nor are the potential implications of the resulting data). • P6, L34, While not the focus of this paper, this is a weak rationale for the hypothetical sensitivity of children to the toxic effects of benzene. The pharmacokinetic angle will not support such a contention. These authors should remove this or actually dig in and provide a real justification for why children might be more susceptible. • P6, L41-53, This section superficially tries to discuss a very complex and important subject. It has no citations, which is certainly not appropriate. Perhaps more importantly, attempting to compare childhood leukemias to adult leukemias in 3 sentences in not

	possible and adds nothing to this manuscript. Which additional genetic changes are required, for example? • P6, In the list of established etiological risk factors for AML, the exposure levels matter. This should be discussed in the "causation' context, particularly in a paper trying to add some badly needed quantification of exposures into the childhood leukemia question. These authors also need to include cigarette smoking in that list, as some investigators believe it is the single most important environmental risk factor for AML [4]. • P11, Results, I found this entire section to be extremely difficult to read. It needs considerable editorial revision. • P12, L56 The observed lack of a relationship between smoking in the household and childhood leukemia risk is really important. Almost all benzene exposure assessments have identified smoking as a major source of benzene exposure, including those only exposed secondhand. Exposure to smoke was clearly evident in the cotinine levels reported in the children. Therefore, they were also exposed to the benzene present in the cigarette smoke. If low levels of benzene before conception, during pregnancy, and in early childhood actually increase the risk of childhood leukemia, it should be evident in smoking households. The literature on smoking and leukemia risk in children is very different between ALL and AML. This robust literature regarding parental smoking should at least be discussed. • P13, L5 Please define "crèche" • P14, L8 This is a very important result (even if this is a pilot study)…probably the most important in the paper. This should have its own section, both here and in the discussion. • P15, L15 This section is difficult to reconcile with previous exposure assessments. Given the low levels of benzene in the ambient air and in cigarette smoke, growing up in a smoking household should have made significant contributions to the levels of benzene measured [5]. As this is not what was reported in this study, this needs to be more fully described. Also, having an attached garage was also important in understanding and characterizing background benzene exposures. • P20, L30 This statement is true for ALL only. I am unaware of any evidence that suggests that childhood AML is linked to any type of infection. The term "childhood leukemia" loses a lot (perhaps all) of its relevance when considering potential etiologies. This should be made very clear in this mansucript. • P21, L30-38 I have no idea what this paragraph means. • P21, L50, This is an important topic and one that needs more description than a single sentence. • P22, L5 What does this "genome-exposome" reference add to this manuscript? The grammar of this sentence is also somewhat off. 1. Schnatter, A.R., et al., Myelodysplastic syndrome and benzene exposure among petroleum workers: an international pooled analysis. J Natl Cancer Inst, 2012. 104(22): p. 1724-37. 2. Hays, S.M., et al., Biomonitoring Equivalents for benzene. Regul Toxicol Pharmacol, 2012. 62(1): p. 62-73. 3. Pyatt, D.W.a.H., S., Age related differences in secondary malignancies in Children: Lessons learned form the pediatric clinical experience. Toxicological Sciences, 2006(S154): p. #154. 4. Services, U.S.D.o.H.a.H., The Health Consequences of Smoking: A Report of the Surgeon General. Atlanta: U.S. Department of

	Health and Human Services, Center for Disease Control and Prevention, National Center for Chronic Disease Preventionm, 2004. 5. Pyatt, D.W., et al., United States Voluntary Children's Chemical Evaluation Program (VCCEP) risk assessment for children exposed to benzene. Toxicol Mech Methods, 2012. 22(2): p. 81-104.

REVIEWER	Dr. Luoping Zhang Associate Adjunct Professor School of Public Health University of California, Berkeley United States of America
REVIEW RETURNED	15-Dec-2012

THE STUDY	In regards to the final question in this section about “supplemental documents”, I was unsure of how to respond. So I will elaborate here: I believe that the information contained in the supplemental materials of this paper is well explained in the manuscript.
GENERAL COMMENTS	This is an important study in the field of benzene exposure in childhood leukemia risk. The authors claim that it is the first pilot study in childhood leukemia and personal benzene exposure, which may be the case. It is also unique in that the authors are able to measure the exact benzene exposure level. Although the study is small, the results seem convincing for larger-scale application. The paper is well written, thoughtful, and concise, and the results, methods, and discussion are presented in a straightforward manner. For these reasons, I recommend the acceptance of this paper.

VERSION 1 – AUTHOR RESPONSE

General comments

1. Brevity is a great effort for southern Europeans, especially Italians. At times, perhaps, we exaggerate. Compatibly with the word limit, we have tried to make the text easier to read.

2. We agree that the pooled analysis of three cohort studies of petroleum workers, recently published by Schnatter et al., is an important paper and that the strong association observed between benzene exposure and myelodysplastic syndrome (MDS) is the most relevant finding. Consequently, we have added a sentence in the Introduction and included the article among the references. However, MDS are considered neoplastic diseases since 2002 only, and are extremely rare in children. For example, over the period 2000-2009, 8 cases of MDS in children aged 0 to 14 years (2.5% of all leukaemias) were recorded by the Paediatric Cancer Registry of the Piedmont Region (Dr. Paola Pisani, personal communication).

3. We acknowledge the relevance of the exposure metric issue. Unfortunately, our pilot study relied on one-week air samples collected by passive samplers, so that the average weekly benzene concentration, and the average yearly concentration over four seasonal one-week samples, were the only metrics available.

4. The referee is right in stressing that our study “really does not inform on the issue of environmental levels of benzene and childhood AML risks (or MDS), which might be the most important question to address”. On the other hand, we deem to have clearly stated that our pilot study was carried out in the

context of an Italian case-control study of leukaemia in children aged 0 to 10 years at diagnosis. In this age range, based on data from the pool of 32 Italian Cancer Registries over the period 2004-2008, the gender-specific incidence rates of childhood leukemias (standardized ever the European population) are 6.1 per 100,000 among males, and 5.1 per 100,000 among females (Dr. Emanuele Crocetti, AIRTUM - Italian Association of Cancer Registries -, personal communication). The corresponding rates of acute lymphoid leukaemia (ALL) are 5 per 100,000 in males and 4.1 per 100,000 in females, whereas the rates of acute myeloid leukaemia (AML) are 0.05 per 100,000 in males and 0.04 per 100,000 in females. As a proportion, AML represents about 8% of all leukaemia cases in Italian children aged 10 years or less . Thus, the number of AML cases in our pilot study (4 out of 43 participant cases) is consistent with that expected. According with the referee's comment, we conclude that the exposure assessment method used in the pilot study could be considered in future studies of benzene and childhood leukemia risk, "with priority given to AML".

5. In agreement with the referee's opinion, we judged the fairly good correlation between S-PMA and benzene exposure "a quite surprising results, considered that S-PMA is believed to represent less than 1% of urinary benzene metabolites for exposures to benzene at air concentrations between 0.1 and 10 ppm". It is certainly true that benzene in blood is a more valid biological index of benzene exposure than any other available biomarker. That notwithstanding, validity is not the only criterion to consider in epidemiological studies of exposure-disease relationships, especially when children are involved. Acceptability to the study subjects and costs of the exposure assessment method are equally, if not more, relevant issues. For these reasons, we deem that blood benzene is not a suitable exposure index in case-control studies of paediatric cancers. Moreover, we are fairly confident that most ethical committees would share such an opinion.

Specific comments

• P4, L7, The term "limited evidence" is written twice
- The mistake has been corrected.

• P4, L9, This sentence is unclear. Exposure to benzene causes what type of leukemia? Also, what exactly are 'relatively high, lifetime environmental exposures'? This sentence as written is difficult to interpret.

- The unclear sentence has been replaced with the following one: "Exposure to benzene would increase the risk of AnLL at levels of lifetime environmental exposure ≥120 ppb".

• P4, L38, The entire concept of correlation with magnetic fields confusing. Why this even being considered? There has been a lot of effort trying to understand the potential relationship between EMF and childhood leukemia risk. Is this why the reader should care about this? The rationale for investigating this potential connection is not clear (nor are the potential implications of the resulting data).

- Our pilot study was carried out in the context of an Italian case-control study investigating, inter-alia, the effect of exposure to extremely low frequency magnetic fields (ELF-MF) on the risk of childhood leukemia. There is still limited evidence for an association between childhood leukemia and exposure to ELF-MF. The association is consistent and apparently specific, but its causality is still questionable. Confounding and participation bias are the most likely alternative explanations. Please see:
World Health Organization. Extremely low frequency fields. Environmental Health Criteria N° 238. Geneva: WHO Press, 2007.
Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). Health effects of exposure to EMF. European Commission – Directorate General for Health & Consumers: Opinion adopted at the 28th plenary on 19 January 2009.

European Health Risk Assessment Network on Electromagnetic Fields Exposure (EFHRAN). Risk analysis of human exposure to electromagnetic fields. Report D2, July 2010.
Kheifets L. et al. Pooled Analysis of Recent Studies on Magnetic Fields and Childhood Leukaemia. Br J Cancer 2010;103:1128-1135.
Schüz J. Exposure to extremely low-frequency magnetic fields and the risk of childhood cancer: update of the epidemiological evidence. Prog Biophys Mol Biol 2011;107 (3):339-342.

• P6, L34, While not the focus of this paper, this is a weak rationale for the hypothetical sensitivity of children to the toxic effects of benzene. The pharmacokinetic angle will not support such a contention. These authors should remove this or actually dig in and provide a real justification for why children might be more susceptible.

- The original sentence has been modified.

• P6, L41-53, This section superficially tries to discuss a very complex and important subject. It has no citations, which is certainly not appropriate. Perhaps more importantly, attempting to compare childhood leukemias to adult leukemias in 3 sentences in not possible and adds nothing to this manuscript. Which additional genetic changes are required, for example?

- The referee is right. Compatibly with the word limit, we have made our best to make the paragraph more incisive and have included the missing quotations.

• P6, In the list of established etiological risk factors for AML, the exposure levels matter. This should be discussed in the "causation' context, particularly in a paper trying to add some badly needed quantification of exposures into the childhood leukemia question. These authors also need to include cigarette smoking in that list, as some investigators believe it is the single most important environmental risk factor for AML [4].

- We agree that for the carcinogenetic effects of benzene, as well as for any other established carcinogens, the exposure levels matters. We also deem to have made that clear in the Introduction of our paper
Instead, based on the following considerations, we do not feel of having to add tobacco smoke in the list of confirmed environmental risk factors for AML presented at page 6 (which already includes benzene).
Active tobacco smoking is an established risk factor for adult acute and chronic myeloid leukemia, and the biological plausibility for a causal relationship of smoking with myeloid leukaemia is provided by the finding of known leukaemogens in tobacco smoke, one of which (benzene) is present in relatively large amounts (IARC. A Review of Human Carcinogens: Personal Habits and Indoor Combustions. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 100E. Lyon: IARC Press, 2012). Parental smoking causes hepatoblastoma in children (ibidem). As to the relationship between second-hand tobacco smoke and childhood leukemia, the body of evidence (2 cohort studies, 27 case-control studies, and 2 meta-analyses) suggests a consistent association of leukaemia (and lymphoma) with paternal smoking preconception and with post-natal combined parental smoking, with risk ratios ranging from 1.5 to 4.0. Maternal tobacco smoking during pregnancy generally showed modest increases in risk, or null or inverse relationships (ibidem).

• P11, Results, I found this entire section to be extremely difficult to read. It needs considerable editorial revision.

- This comment confuses us, because it contrasts with the opinion of the second referee ("The paper is well written, thoughtful, and concise, and the results, methods, and discussion are presented in a straightforward manner"). We felt that a different familiarity with epidemiology may explain the

contrast of opinions and we have not made the suggested “considerable editorial revision”.

• P12, L56 The observed lack of a relationship between smoking in the household and childhood leukemia risk is really important. Almost all benzene exposure assessments have identified smoking as a major source of benzene exposure, including those only exposed secondhand. Exposure to smoke was clearly evident in the cotinine levels reported in the children. Therefore, they were also exposed to the benzene present in the cigarette smoke. If low levels of benzene before conception, during pregnancy, and in early childhood actually increase the risk of childhood leukemia, it should be evident in smoking households. The literature on smoking and leukemia risk in children is very different between ALL and AML. This robust literature regarding parental smoking should at least be discussed.

- The relationship between childhood leukemia and parental smoking was not addressed in our pilot study. At page 12 - line 56 we report that “a higher proportion of controls than cases had both parent smoking”. This finding might be due to a control participation bias, but no empirical evidence is available to us to confirm or disproof such a hypothesis.

• P13, L5 Please define “crèche”

- In the revised version of the manuscript we have replaced crèche with day-care.

• P14, L8 This is a very important result (even if this is a pilot study)…probably the most important in the paper. This should have its own section, both here and in the discussion.

- We did not follow this suggestion because our pilot study does not have the statistical power required to assess the association between childhood leukemia and personal benzene exposure.

• P15, L15 This section is difficult to reconcile with previous exposure assessments. Given the low levels of benzene in the ambient air and in cigarette smoke, growing up in a smoking household should have made significant contributions to the levels of benzene measured [5]. As this is not what was reported in this study, this needs to be more fully described. Also, having an attached garage was also important in understanding and characterizing background benzene exposures.

- At page 15 – line 15 we describe the findings of the analyses aimed at evaluating the predicting role of a number of potential determinants on the level and variability of personal exposure to benzene among the children participating in our pilot study. The “Results” section is not the appropriate place to discuss the findings. One possible explanation for the apparent trivial influence of exposure to second-hand tobacco smoke on personal benzene exposure was residual confounding from lack of samples collected in one centre other than in summer. In facts, as reported at page 15-lines 52-54, in the restricted data-set of at least two series of measurements in different seasonal periods (cold and warm) “independent effects of both outdoor benzene and urinary cotinine levels on personal benzene exposure were observed”.
Multistore buildings are the most common types od dwelling in Italy, thus Italian children do not usually play in house-attached garages.

• P20, L30 This statement is true for ALL only. I am unaware of any evidence that suggests that childhood AML is linked to any type of infection. The term “childhood leukemia” loses a lot (perhaps all) of its relevance when considering potential etiologies. This should be made very clear in this mansucript.

- The referee is right. We have changed the text accordingly.

• P21, L30-38 I have no idea what this paragraph means.

- Please, see our answer to the comment related to P4, L38.

• P21, L50, This is an important topic and one that needs more description than a single sentence.

- We agree, but the unsuccessful conclusion of the day-to-day variability sub-study, along with the word limit, prevent us from following this suggestion.

• P22, L5 What does this “genome-exposome” reference add to this manuscript? The grammar of this sentence is also somewhat off.

- Actually, we deem that the final sentence of the manuscript is more appropriate today than it was at the time of the paper’s submission. Two large research projects (Exposomics and HELIX) have been jointly launched on 12 November 2012, addressing complementary aspects of the “exposome” (i.e. the sum of all of the environmental components, including lifestyle factors and chemicals we are exposed to, that influence our health over the course of a lifetime); this joint launch marks the EU’s biggest investment in environmental health research to date (IARC Press Release 214/2012; http://www.iarc.fr/en/media-centre/pr/2012/pdfs/pr214_E.pdf). We have updated the relevant quotations.

VERSION 2 – REVIEW

REVIEWER	David Pyatt, PhD Principal and co-counder Summit Toxicology, LLP University of Colorado, SOP, SPH
REVIEW RETURNED	21-Jan-2013

- The reviewer completed the checklist but made no further comments.